Puget Sound Water Column Endocrine Disrupting Compounds Survey

Sampling and Analysis Plan

Prepared for the
King County Department of Natural Resources and Parks
Wastewater Treatment Division

Prepared by the
King County Department of Natural Resources and Parks
Marine and Sediment Assessment Group

March 2003

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Prepared by
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March 2003

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1 INTRODUCTION

This sampling and analysis plan (SAP) presents project information and sampling and analytical methodologies that will be employed to perform a survey of endocrine disrupting compounds in the Puget Sound marine water column. The SAP includes a description of the project, survey design, sampling and analytical methodologies, and project reporting. This work is being performed as part of the King County Wastewater Treatment Division's ongoing efforts to monitor the health of the marine environment in Puget Sound.

1.1 Project Background

Endocrine disrupting compounds (EDCs) are chemicals that mimic natural hormones, inhibit the action of hormones, or alter normal regulatory functions of the immune, nervous, and endocrine systems (King County, 2002). Municipal wastewater treatment systems are typically viewed as a potential "line of defense" against the release of EDCs to waterways. Although most chemicals are removed through primary or secondary treatment processes, some portion of these chemicals may be discharged in treated effluent.

A variety of potentially endocrine disrupting chemicals are known or suspected to be present in secondary treated wastewater effluent. These chemicals include natural and synthetic hormones, alkylphenolic compounds, phthalates, pesticides, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and some metals. The purpose of this survey is to begin characterizing concentrations of EDCs in the ambient waters of Puget Sound, Elliott Bay, and the lower Duwamish River. Separate studies, not included in the scope of this SAP, will characterize EDCs in several freshwater bodies in King County.

This survey will involve the collection and analysis of ambient Puget Sound water samples from several locations. Samples will be collected and analyzed on a quarterly basis for a period of one year. Analyses will include both standard, instrumental methods for a wide variety of trace organic compounds and immunoassay testing for two hormonal compounds. Analysis of metals is not included in this survey. The distribution of trace metals in Puget Sound waters was fully characterized in a previous King County study, conducted in 1999 and 2000 (King County, 2001).

1.2 Project Organization and Schedule

The tasks involved in conducting the Puget Sound Water Column Endocrine Disrupting Compounds Survey and the King County personnel who will assume responsibility for those tasks are listed below.

- **Scott Mickelson** – Marine & Sediment Assessment Group scott.mickelson@metrokc.gov 206-296-8247. Project management, survey design, preparation of SAP, data validation and analysis, and preparation of final survey report.
- **Jean Power** – Environmental Laboratory jean.power@metrokc.gov 206-684-2393. Coordination of field activities including preparation of sampling equipment and collection of samples.
- **Diane McElhany** – Environmental Laboratory diane.mcelhany@metrokc.gov 206-684-2304. Coordination of trace organic laboratory analyses.
- **Jim Buckley** – Environmental Laboratory jim.buckley@metrokc.gov 206-684-2314. Coordination and analysis for immunoassay testing.

- **Katherine Bourbonais** – Environmental Laboratory katherine.bourbonais@metrokc.gov 206-684-2382. Coordination of all Environmental Laboratory activities, data validation, and data reporting.
- **Betsy Cooper** – Wastewater Treatment Division betsy.cooper@metrokc.gov 206-263-3728. Review of survey design, SAP, and final survey report and coordination of King County's EDC technical workgroup.

The anticipated project schedule is:

- February 2003 – Preparation of sampling and analysis plan.
- March 2003 – First quarterly sampling event.
- June 2003 – Second quarterly sampling event.
- August 2003 – Review of analytical results from first two quarterly sampling events.
- September 2003 – Third quarterly sampling event.
- December 2003 – Fourth and final quarterly sampling event.
- February/March 2004 – Data validation, review, and analysis.
- April 2004 – Preparation of final survey report.

2 SURVEY AREA DESCRIPTION

The survey area encompasses the Central Basin of Puget Sound, Elliott Bay, and the lower Duwamish River (Figure 1).

Puget Sound is a fjord-like estuary that extends approximately 230 kilometers in a north-south direction. The average depth in Puget Sound is 106 meters, however, depths in the Central Basin can reach greater than 280 meters. Although Puget Sound receives fresh water from many rivers and streams, it maintains near-oceanic salinity throughout the year. Many complex factors affect water quality in Puget Sound including water currents, physical and chemical processes, and human activities. Urbanization and subsequent population growth around the Central Basin have increased anthropogenic inputs to the Sound from storm water runoff, industrial discharges, combined sewer overflows, treated wastewater effluent, and atmospheric deposition.

Elliott Bay, approximately 21 square kilometers in area, forms the western boundary of the commercial core of Seattle. Land use on the waterfront surrounding the bay is mainly marine-oriented industrial and urban commercial with marine traffic on the bay heavy at all times of the year. The bay opens to the Central Basin of Puget Sound to the east. Depths in open Elliott Bay range from 150 to 180 meters while depths near the Seattle waterfront are in the range of 10 to 20 meters. The open waters of Elliott Bay are dominated by Puget Sound marine water masses with a fresh water lens from the Duwamish River occupying the upper five meters. Natural shorelines with intertidal zones are present along the northeast and southwest shores of the bay. Piers, a sea wall, and rip-rapping have replaced natural shorelines along the waterfront in the commercially developed areas of the bay.

The lower Duwamish River is a highly industrialized, salt-wedge estuary influenced both by river flow and tidal effects. At its mouth, the river splits into the East and West Waterways, flowing around Harbor Island into Elliott Bay. The river is considered an estuarine system, exhibiting both marine and fresh water characteristics. The lower portion of the Duwamish River has been straightened, dredged, and rip-rapped to facilitate navigation and commerce. River depths range from approximately 17 meters (m) near the mouth to less than a meter in some areas, depending on tidal effects.

3 SURVEY DESIGN

The primary goals of the Puget Sound Water Column Endocrine Disrupting Compound Survey are to:

- survey and characterize the nature of potentially endocrine disrupting compounds in ambient waters of Puget Sound, Elliott Bay, and the Duwamish River; and
- evaluate spatial and temporal variations of those compounds that are detected.

A secondary goal is to validate the use of immunoassay testing both as a weight-of-evidence approach for evaluating sample results generated by standard, instrument analysis and to assess the potential of using these relatively low-cost, rapid assays as a reliable tool for measuring concentrations of hormonal compounds in natural waters.

3.1 Data Quality Objectives

The data quality objectives (DQOs) are to collect data of sufficient quantity and quality to meet the survey goals. Statistical analysis of data will be performed to evaluate whether a sufficient quantity of data has been collected to meet the survey goals.

The survey goals are to characterize water concentrations of various trace organic compounds at different locations and depths and to evaluate any differences between sites and depths, either spatially or temporally. It is anticipated that many organic compounds will not be detected in ambient Puget Sound water. Statistical analysis of data that are generally "undetected" will use binomial calculations on the probability of finding a sample with a detectable concentration of the organic compound and the probability of finding two and three samples in succession with detectable values at a given site or depth. Statistical analysis of data for those organic compounds that are detected regularly or occasionally will be accomplished through the use of medians and interquartile ranges.

Validation of project data will assess whether the data collected are of sufficient quality to meet the survey goals. The data quality issues of precision, accuracy, bias, representativeness, completeness, and comparability are described in the following sections.

3.1.1 Precision, Accuracy, and Bias

Precision is the agreement of a set of results among themselves and is a measure of the ability to reproduce a result. Accuracy is an estimate of the difference between the true value and the determined mean value. The accuracy of a result is affected by both systematic and random errors. Bias is a measure of the difference, due to a systematic factor, between an analytical result and the true value of an analyte. Precision, accuracy, and bias for analytical chemistry and immunoassay testing may be measured by one or more of the following quality control (QC) procedures:

- collection and analysis of field replicate samples (field replicate results should exhibit a relative percent difference less than 50% in order for the evaluation of the spatial and temporal chemical concentrations to be meaningful); and
- analysis of various laboratory QC samples such as blanks, spikes, and replicates.

3.1.2 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point, or an environmental condition. Water samples will be collected from stations with predetermined coordinates and sampling depths to represent specific site conditions, both compared to other locations and at each location over time.

3.1.3 Completeness

Completeness is defined as the total number of samples analyzed for which acceptable analytical data are generated, compared to the total number of samples submitted for analysis. Sampling at stations with known position coordinates in favorable conditions, along with adherence to standardized sampling and testing protocols will aid in providing a complete set of data for this survey. The goal for completeness is 100%. If 100% completeness is not achieved, the project team will evaluate if the DQOs can still be met or if additional samples may need to be collected and analyzed.

3.1.4 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. This goal is achieved through using standard techniques to collect and analyze representative samples, along with standardized data validation and reporting procedures. By following the guidance of this SAP, the goal of comparability between sampling events will be achieved. Historical water quality data from the survey area may be compared with data generated from this survey to enhance data analysis efforts. Previous data will be used if comparable sampling and analytical techniques were employed.

3.2 Sampling Stations and Selection Rationale

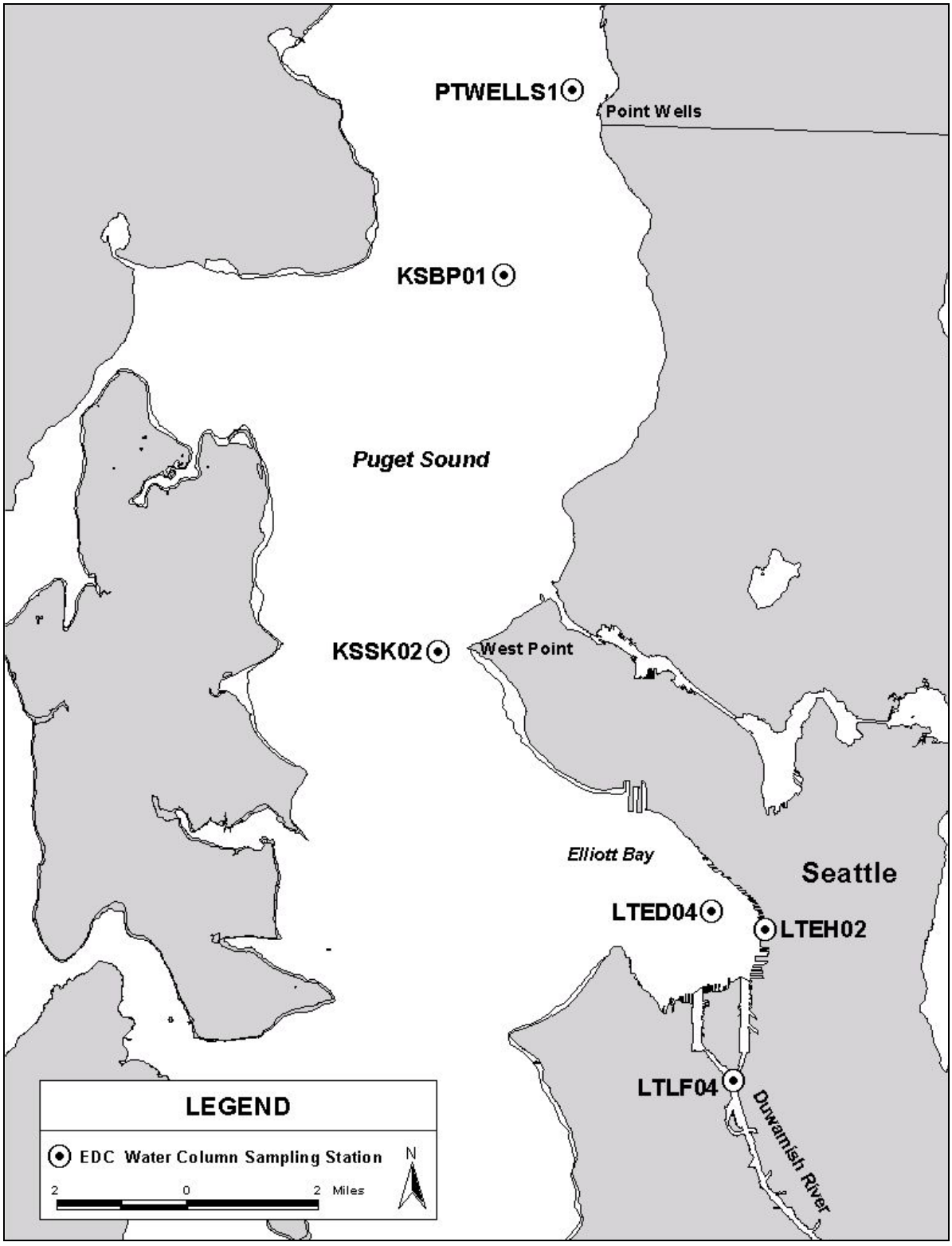
Sampling stations and depths were chosen to provide the opportunity to characterize water concentrations of EDCs in a variety of Puget Sound environments, both proximal and distal to anthropogenic inputs, and vertically through the water column. Samples will be collected from six stations, located in the Central Basin of Puget Sound, Elliott Bay, and the Duwamish River (Figure 1).

This survey will not include discrete sampling of the surface microlayer itself, however, a surface sample will be collected at each station that will incorporate the surface microlayer in the sample. Additional depths will be collected at five of the six stations. The additional sampling depths at three of the four open-water stations were set at: a mid-range depth of 50 meters (m); and a variable deeper depth, dependent on total depth of the water column at the station. Station names, locations, coordinates, and sampling depths are shown in Table 1.

Table 1
Puget Sound Water Column EDC Survey
Sampling Stations

Station Name	Location	Coordinates (NAD83)	Sampling Depths
PTWELLS1	Central Basin near Point Wells	290385N, 1253569E	Surface, 50 m, 120 m
KSBP01	Central Basin	275439N, 1248062E	Surface, 50 m, 210 m
KSSK02	Central Basin near West Point	245121N, 1242740E	Surface, 25 m, 70 m
LTED04	Elliott Bay	224199N, 1264780E	Surface, 50 m, 75 m
LTEH02	Elliott Bay Waterfront	222696N, 1269069E	Surface
LTLF04	Duwamish River	210509N, 1266494E	Surface, 1 m above bottom

Figure 1
Puget Sound Water Column EDC Survey
Sampling Locations



- **PTWELLS1** is located in the Central Basin of Puget Sound, near Point Wells on the eastern shore. This station was chosen to represent Puget Sound *baseline* conditions in the vicinity of the planned outfall location for King County's new Brightwater regional wastewater treatment system. Three samples will be collected at this station; at the surface, from the 50-meter mid-range depth, and from a deepest depth of 120 meters.
- **KSBP01** is located in the Central Basin of Puget Sound, midway between Point Jefferson on the Kitsap Peninsula and northern King County. This station was chosen to represent *ambient* conditions in Puget Sound, away from all direct anthropogenic inputs. Three samples will be collected at this station; at the surface, from the 50-meter mid-range depth, and from a deepest depth of 210 meters.
- **KSSK02** is located at the outfall for King County's West Point secondary wastewater treatment plant. This station was chosen to represent conditions in Puget Sound at the location of a direct, treated wastewater effluent input. Three samples will be collected at this station; at the surface, from a mid-range depth of 25 meters at which effluent may be occasionally trapped, and from a deepest depth of 70 meters.
- **LTED04** is located in central Elliott Bay. This location was chosen to represent conditions in Elliott Bay, away from most *direct* anthropogenic inputs. Three samples will be collected at this station; at the surface, from the 50-meter mid-range depth, and from a deepest depth of 75 meters.
- **LTEH02** is located along the Elliott Bay waterfront, at Pier 48. This location was chosen to represent conditions in Elliott Bay along the commercial shoreline, which receives inputs from various commercial dischargers, storm water, combined sewer overflows, and the Duwamish River. One sample will be collected at this station, from the surface.
- **LTLF04** is located in the Duwamish River, just south (upstream) of Harbor Island. This location was chosen to represent conditions in the Duwamish River, prior to entering Elliott Bay, downstream of inputs from numerous industrial dischargers, storm water, and combined sewer overflows. Two samples will be collected at this station; at the surface from outflowing fresh river water, and at a depth of one meter above the river bottom, from marine water introduced by the incoming salt wedge.

King County has previously collected and analyzed samples for trace organic compounds from stations PTWELLS1, KSBP01, and KSSK02. King County also has long-term, general water quality data from stations KSBP01, KSSK02, and LTEH02.

3.3 Sampling Frequency

Samples will be collected on a quarterly basis in March, June, September, and December of 2003. Specific weather conditions or events have not been targeted in order to maintain a random sampling design specifically for the collection of ambient samples during four seasons. Inclement weather conditions that preclude sampling on a scheduled day, however, may result in rescheduling the sampling event later in the designated month.

3.4 Analytical Parameters

Analytical parameters for this survey were chosen based on an initial survey of national and international target chemicals under study as suspected EDCs, literature reviews (Birkett and Lester, 2003; Davis et al, 1999; King County 2002), discussions with staff of the National Marine Fisheries Service and U.S. Fish and Wildlife Service, and analytical capabilities of the King County Environmental Laboratory.

Trace organic instrument analysis will include the following parameters:

- chlorinated pesticides;
- other pesticides (Atrazine and Vinclozolin);
- polychlorinated biphenyls (PCBs);
- PAH compounds (both low (LPAH) and high (HPAH) molecular weight);
- phthalates;
- hormonal compounds; and
- phenols.

Immunoassay testing will be performed for two hormonal compounds, estradiol and ethinylestradiol.

The majority of analytical parameters for this survey were chosen due to their purported endocrine disrupting potential. The complete list of analytical parameters includes some compounds not considered to be EDCs, however, this analytical suite was designed to encompass both this survey and other, concurrent King County studies as well as to characterize these *ancillary* compounds in water samples that include the surface microlayer. Tables 2 and 3 summarize the target EDC and ancillary analytical parameters.

Table 2
Puget Sound EDC Survey Analytical Parameters
Endocrine Disrupting Compounds List

EDC	Category	EDC	Category
Estradiol	Hormone	Aldrin	Pesticide
Estrone	Hormone	Atrazine	Pesticide
Ethinylestradiol	Hormone	Gamma-BHC (Lindane)	Pesticide
Methyltestosterone	Hormone	Alpha-Chlordane	Pesticide
Progesterone	Hormone	Gamma-Chlordane	Pesticide
Testosterone	Hormone	4,4'-DDE	Pesticide
Acenaphthene	LPAH	4,4'-DDT	Pesticide
Acenaphthylene	LPAH	Dieldrin	Pesticide
Anthracene	LPAH	Endosulfan I	Pesticide
Benzo(a)anthracene	HPAH	Endosulfan II	Pesticide
Benzo(a)pyrene	HPAH	Endrin	Pesticide
Benzo(b)fluoranthene	HPAH	Heptachlor	Pesticide
Benzo(k)fluoranthene	HPAH	Heptachlor Epoxide	Pesticide
Benzo(g,h,i)perylene	HPAH	Hexachlorobenzene	Pesticide
2-Chloronaphthalene	LPAH	Methoxychlor	Pesticide
Chrysene	HPAH	Vinclozolin	Pesticide
Dibenzo(a,h)anthracene	HPAH	Bisphenol A	Phenol
Fluoranthene	HPAH	2,4-Dichlorophenol	Phenol
Fluorene	LPAH	Pentachlorophenol	Phenol
Indeno(1,2,3-c,d)pyrene	HPAH	2,4,6-Trichlorophenol	Phenol
2-Methylnaphthalene	LPAH	Bis(2-ethylhexyl) Adipate	Plasticizer
Naphthalene	LPAH	Bis(2-ethylhexyl) Phthalate	Plasticizer
Phenanthrene	LPAH	Butyl Benzyl Phthalate	Plasticizer
Pyrene	HPAH	Diethyl Phthalate	Plasticizer
PCB Aroclors®	PCB	Di-n-butyl Phthalate	Plasticizer
Total 4-Nonylphenol	Surfactant		

Table 3

Puget Sound EDC Survey Analytical Parameters

Ancillary Compounds List

Ancillary Compound	Category	Ancillary Compound	Category
Alpha-BHC	Pesticide	1,2-Dichlorobenzene	Chlorobenzene
Beta-BHC	Pesticide	1,3-Dichlorobenzene	Chlorobenzene
Delta-BHC	Pesticide	Caffeine	Sewage Tracer
4,4'-DDD	Pesticide	1,4-Dichlorobenzene	Sewage Tracer
Endosulfan Sulfate	Pesticide	Carbazole	PAH Tracer
Endrin Aldehyde	Pesticide	Dibenzofuran	PAH Tracer
Toxaphene	Pesticide	Phenol	Phenol

4 SAMPLE COLLECTION AND HANDLING

The representativeness of a data set may be enhanced by following a standard set of protocols for collecting environmental samples. This section describes methodologies and protocols for the collection of representative water samples from the offshore water column in Puget Sound, Elliott Bay, and the Duwamish River, and the nearshore environment along the Seattle waterfront, specifically for the analysis of trace organic compounds. All samples will be collected by staff of the King County Environmental Laboratory's Environmental Services Section.

4.1 Onshore Sample Collection

Samples will be collected from shore at one station in Elliott Bay, LTEH02, located on the Seattle waterfront at Pier 48. Samples will be collected only from the water's surface at this station, using a stainless-steel bucket lowered from the pier on a cotton or other non-plastic rope. A field blank will be collected at this station during the onshore portion of each sampling event, according to procedures outlined in Section 4.4.

4.1.1 Sampling Equipment Preparation

A stainless-steel sampling bucket and stainless-steel funnel will be cleaned prior to each sampling event according to the following procedures, in the order listed.

1. Wash the bucket and funnel in detergent and tap water and rinse thoroughly with tap water.
2. Rinse the bucket and funnel with deionized, reagent, laboratory water.
3. Rinse the bucket and funnel with solvents in the following order: methanol, acetone, and methylene chloride.
4. Wrap the bucket and funnel in clean aluminum foil.

New, unused, cotton or other non-plastic rope will be used for each sampling event. The rope will be removed from any plastic wrapping and stored wrapped in clean aluminum foil until use.

4.1.2 Sample Collection

Prior to collecting the surface water sample, a field blank will be collected from the dedicated, onshore stainless-steel bucket and funnel during each sampling event according to procedures outlined in Section 4.4.1. The surface water sample will be collected by lowering the bucket and allowing it to fill, taking care to incorporate the actual surface of the water into the sample. The bucket will then be retrieved and the water sample poured through the funnel into three (3) 1-liter, amber, glass bottles and two (2) 40-milliliter (ml), amber, glass VOA vials. Sampling personnel will note the date and time of sample collection and whether the sampling event occurred during "storm" (actively raining at the time of sample collection) or "non-storm" conditions.

4.2 Offshore Sample Collection

Offshore water samples will be collected from three open-water Puget Sound stations (KSBP01, PTWELLS1, and KSSK02; three depths at each), one Elliott Bay station (LTED04; three depths), and one Duwamish River station (LTLF04; two depths). Samples will be collected from the water's surface at each station, using a stainless-steel bucket and funnel. Samples from the other discrete depths at each station will be collected with Niskin®

sample-collection bottles, deployed via hydrowire and hydraulic winch from King County's research vessel *Liberty*. Two field blanks and one field replicate will be collected during the offshore portion of each sampling event.

4.2.1 Sampling Equipment Preparation

A separate stainless-steel bucket and stainless-steel funnel will be cleaned prior to each sampling event according to the procedures outlined in Section 4.1.1. Two (2) Niskin® bottles will also be employed to collected subsurface water samples. The closure mechanism on a Niskin® bottle employs flexible tubing, several types of which can potentially impart phthalate compounds to a water sample. The King County Environmental Laboratory has analyzed several types of flexible tubing for phthalate content. Due to the importance of unbiased sampling to this survey, a tubing will be used that has the lowest phthalate content possible while still exhibiting acceptable performance as a closure mechanism for the Niskin® bottle.

4.2.2 Station Positioning

A precise method of offshore station positioning is important for surveys in which sampling stations are revisited multiple times. This survey will not only assess spatial differences in water-column chemical constituents over the survey area but temporal differences at each particular location as well. In order to assess temporal differences in water-column chemical constituents, the stations must be revisited as precisely as possible.

Station positioning for this survey will be accomplished using a Differential Global Positioning System (DGPS). Prior to the first sampling event, prescribed station coordinates (State Plane NAD83 coordinate system) will be loaded into the shipboard DGPS. During sampling events, the shipboard navigational system will utilize the differential data transmissions from regional Coast Guard base stations to automatically correct its GPS satellite data. The GPS antenna is boom-mounted above the sampler descent line to achieve a more accurate coordinate fix above the sampling point. Previous DGPS usage indicates that an average precision of five meters can usually be attained.

4.2.3 Sample Collection

The surface water sample at each of the five offshore station will be collected according to the procedures outlined in Section 4.1.2. Prior to sample collection at each of the second through fifth offshore stations, the bucket and funnel will be thoroughly rinsed with ambient water at the station. A field blank will also be collected from the dedicated offshore stainless-steel bucket and funnel during each sampling event, prior to sample collection (see Section 4.4.1). An additional five (5) 1-liter, amber, glass bottles will be filled for the surface water sample collected at station KSBP01 (a total of eight (8) bottles) to provide sufficient sample matrix for analysis of the trace organics' matrix spike and matrix spike duplicate QC samples. The additional sample matrix will be collected from this station during every sampling event.

A field blank will be collected from one of the Niskin® bottles during each sampling event according to procedures outlined in Section 4.4.1, prior to collecting the first subsurface water-column sample. Subsurface water-column samples will be collected by attaching the Niskin® bottles to the hydrowire and lowering them to the prescribed depth, using the meter wheel on the hydraulic winch. At the appropriate depths, a tripping mechanism is deployed

to close the Niskin® bottles, entraining the sample matrix. Upon retrieval, samples will be poured directly into the appropriate containers (three (3) 1-liter, amber, glass bottles and two (2) 40-ml, amber, glass VOA vials per sample).

Sampling personnel will note the date and time of sample collection and whether the sampling event occurred during "storm" (actively raining at the time of sample collection) or "non-storm" conditions.

4.3 Sample Handling

All samples will be kept in ice-filled coolers until delivery to the King County Environmental Laboratory. Upon receipt, all samples will be refrigerated to maintain a temperature of approximately 4° Celsius until analysis. All samples will be analyzed within method-specific holding times. Trace organic extractions will be completed within 7 days of sample collection and instrument analysis will be completed within 40 days of sample extraction. Immunoassay tests will be completed within 11 days of sample collection.

4.4 Field Quality Assurance/Quality Control

A strong field quality assurance/quality control (QA/QC) program that includes both standardized sampling protocols and the collection of field QC samples enhances the ability of the resulting data set to meet survey DQOs. The primary goal of the field sampling effort is to collect samples that are as free as possible of introduced contamination by target analytes. Several steps will be taken to minimize the potential for contamination and cross-contamination of samples.

- Sampling personnel wear personal protective equipment that includes chemical-resistant gloves as part of King County's overall field safety program. The protective gloves can, however, potentially introduce phthalate compounds to samples that would be readily detectable during laboratory analysis. The King County Environment Laboratory has analyzed many types of gloves for phthalate content. Sampling personnel for this project will wear chemical-resistant gloves that have the lowest potential for introducing phthalate compounds into the water samples.
- Many of the target analytes for this project are found in diesel exhaust. Care will be taken during all offshore sampling events to position the vessel so that contact between the water samples and airborne diesel exhaust is minimized. The vessel will also be positioned to avoid any contact between sampling equipment and diesel exhaust that may have settled onto the water's surface (i.e., a noticeable sheen).
- Cross-contamination of samples may be minimized by collecting them at stations in order of "cleanliness," such as from the station with the least potential for impacts from anthropogenic sources to the station with the highest potential. Samples will be collected in the following station order:
    1. KSBP01 – Puget Sound Central Basin;
    2. PTWELLS1 – Puget Sound near Point Wells;
    3. KSSK02 – Puget Sound, at the West Point wastewater treatment plant outfall;
    4. LTED04 – central Elliott Bay; and
    5. LTLF04 – the Duwamish River.

4.4.1 Field Blanks

A field blank is a sample of analyte-free, reagent water that is supplied by the trace organics laboratory and transported to the sampling site in the same type of bottle used for analytical samples. The sample container is opened at the sampling location and the reagent water transferred to another analytical sample container, employing the same equipment and procedures used in sample collection. The field blank will be analyzed for all of the analytes for which the samples are being analyzed. Analysis of field blanks is used to measure and document any sample contamination resulting from exposure to ambient environmental conditions at the sampling location as well as the effectiveness of the sampling equipment cleaning procedures.

Field blanks will be collected at both the onshore sampling station, LTEH02, using the dedicated, onshore stainless-steel bucket and funnel, and at the first offshore sampling station, KSBP01, using both the dedicated, offshore stainless-steel bucket and funnel and one of the Niskin® bottles. Field blanks will be submitted for both trace organic instrument analysis and immunoassay testing.

The onshore field blank will be collected prior to any sample-collection activities by pouring the laboratory reagent water into the pre-cleaned, stainless-steel bucket. The contents of the bucket will then be poured into three (3) 1-liter, amber, glass bottles and two (2) 40-ml VOA vials through the pre-cleaned, stainless-steel funnel.

The offshore field blank will be collected prior to any sample-collection activities by pouring the laboratory reagent water into one of the Niskin® bottles. The contents of the Niskin® bottle will then be poured directly into three (3) 1-liter, amber, glass bottles and two (2) 40-ml VOA vials. The offshore bucket and funnel field blank will be collected using the same procedure that describes collection of the onshore field blank.

4.4.2 Field Replicates

A field replicate is a second sample collected at a sampling station employing the same equipment and procedures used to obtain the first sample. The field replicate will be analyzed for same suite of analytes as the replicated sample. Analysis of field replicates is used to measure and document the repeatability of sample collection methodologies as well as provide data to assess environmental variability at the sampling station.

One field replicate will be collected during each sampling event at the stations and depths noted in Table 4. The rotation of locations for collecting field replicates will allow an assessment of environmental variability at multiple locations.

Table 4
Puget Sound Water Column EDC Survey
Field Replicate Collection Schedule

Sampling Event	Location	Depth	Analyses
March 2003	KSSK02	25 meters	All organics and immunoassay tests.
June 2003	KSBP01	Surface	All organics and immunoassay tests.
September 2003	LTED04	75 meters	All organics and immunoassay tests.
December 2003	LTLF04	Surface	All organics and immunoassay tests.

Prior to collection of the field replicate, sampling personnel will verify the *Liberty's* current position with the skipper and, if necessary, the vessel will be repositioned back on station. Field replicates for surface water samples will entail a second deployment of the stainless-steel bucket and repetition of the associated sample collection procedures. Field replicates for subsurface water samples will entail a second deployment of the same Niskin® bottle used to collected the sample being replicated. The Niskin® bottle will be deployed to the appropriate depth and repetition of the associated sample collection procedures will be completed.

5 TRACE ORGANIC LABORATORY ANALYSIS

The completeness and comparability of a data set is enhanced by following a standard set of protocols for analyzing samples. Analysis of a prescribed set of laboratory QC samples will also allow a data set to be evaluated in terms of precision, accuracy, and bias. This section describes trace organic analytical methodologies, associated QC protocols, and detection limits. The method detection limit (MDL) is that concentration at which an analyte *can reliably be detected.* The reporting detection limit (RDL) is that concentration at which an analyte *can reliably be quantified.* All detection limits are shown in units of micrograms per liter (μg/L).

5.1 Chlorinated Pesticides/PCBs

Chlorinated pesticide/PCB sample preparation will be performed according to EPA Method 3520C (SW 846 [EPA, 1986]), which is a continuous liquid-liquid extraction technique. About one liter of sample is extracted with approximately 400 ml of methylene chloride for 18 to 24 hours. The sample extract is split, for use in analysis of both chlorinated pesticides/PCBs and miscellaneous endocrine disrupting compounds (see Section 5.3). The chlorinated pesticide/PCB split is dried with sodium sulfate and concentrated to a 1-ml effective final volume. An alumina cleanup is performed on the split according to EPA Method 3610 (SW 846). Chlorinated pesticide/PCB sample analysis will be performed according to EPA Method 608 (SW846), which uses gas chromatography with an electron capture detector (GC-ECD). Table 5 lists the target chlorinated pesticide/PCB analytes and their respective detection limits.

Table 5
Target Chlorinated Pesticide/PCB Analytes and Detection Limits (μg/L)

Chlorinated Pesticide/PCB	MDL	RDL
Aldrin	0.005	0.01
Alpha-BHC	0.005	0.01
Beta-BHC	0.005	0.01
Delta-BHC	0.005	0.01
Gamma-BHC (Lindane)	0.005	0.01
Alpha-Chlordane	0.005	0.01
Gamma-Chlordane	0.005	0.01
4,4'-DDD	0.005	0.01
4,4'-DDE	0.005	0.01
4,4'-DDT	0.005	0.01
Dieldrin	0.005	0.01
Endosulfan I	0.005	0.01
Endosulfan II	0.005	0.01
Endosulfan Sulfate	0.005	0.01
Endrin	0.005	0.01
Endrin Aldehyde	0.005	0.01
Heptachlor	0.005	0.01
Heptachlor Epoxide	0.005	0.01
Methoxychlor	0.025	0.05
Toxaphene	0.050	0.10
PCB Aroclors®	0.050	0.10

5.2 Base/Neutral/Acid Extractable Semivolatile Compounds (BNAs) and Atrazine

Sample preparation for BNAs, which will include Atrazine (a triazine pesticide), will be performed according to EPA Method 3520C, described in Section 5.1. The extraction will be performed on a separate 1-liter volume of sample matrix, however, no sample cleanup will be necessary. BNA/Atrazine sample analysis will be performed according to EPA Method 8270C (SW846), which uses gas chromatography with mass spectroscopy (GC-MS), retrofitted with a large volume injector (LVI) to lower the detection limits. Table 6 lists the target BNA/Atrazine analytes and their respective detection limits.

Table 6
Target BNA Analytes (including Atrazine)
and Detection Limits (µg/L)

BNA Compound	MDL	RDL
Acenaphthene	0.010	0.050
Acenaphthylene	0.010	0.050
Anthracene	0.010	0.050
Atrazine (Triazine Pesticide)	0.050	0.100
Benzo(a)anthracene	0.025	0.050
Benzo(a)pyrene	0.010	0.025
Benzo(b)fluoranthene	0.010	0.025
Benzo(g,h,i)perylene	0.100	0.250
Benzo(k)fluoranthene	0.010	0.025
Benzyl Butyl Phthalate	0.010	0.025
Bis(2-Ethylhexyl)Phthalate	0.010	0.025
Caffeine	0.010	0.025
Carbazole	0.025	0.050
2-Chloronaphthalene	0.010	0.050
Chrysene	0.025	0.050
Dibenzo(a,h)anthracene	0.100	0.250
Dibenzofuran	0.010	0.025
1,2-Dichlorobenzene	0.050	0.250
1,3-Dichlorobenzene	0.050	0.250
1,4-Dichlorobenzene	0.050	0.250
2,4-Dichlorophenol	0.500	1.000
Diethyl Phthalate	0.010	0.025
Di-N-Butyl Phthalate	0.010	0.025
Fluoranthene	0.010	0.025
Fluorene	0.010	0.025
Hexachlorobenzene	0.025	0.050
Indeno(1,2,3-Cd)Pyrene	0.100	0.250
2-Methylnaphthalene	0.100	0.500
Naphthalene	0.025	0.050
Pentachlorophenol	0.500	1.000
Phenanthrene	0.010	0.025
Phenol	0.500	1.000
Pyrene	0.010	0.025
2,4,6-Trichlorophenol	0.500	1.000

5.3 Miscellaneous Endocrine Disrupting Compounds

The "miscellaneous endocrine disrupting compounds" include a pesticide (Vinclozolin), three BNA compounds, and six hormones (see Table 7). Sample preparation for these analytes will be performed as described in Section 5.1. After splitting the extract, the miscellaneous endocrine disrupting compound split will be water-washed as a cleanup procedure. Sample analysis for these compounds will be performed by GC-MS with LVI, operated in the Selected Ion Monitoring (SIM) mode. Table 7 lists the target miscellaneous endocrine disrupting compounds and their respective detection limits.

Table 7
Miscellaneous Endocrine Disrupting Compounds and Detection Limits (µg/L)

ED Compound	MDL	RDL
Bis(2-ethylhexyl)adipate**	0.100	0.500
Bisphenol A**	0.100	0.500
Estradiol***	0.010	0.025
Estrone***	0.010	0.025
Ethynyl estradiol***	0.010	0.025
Methyltestosterone***	0.010	0.025
4-Nonylphenol (total)**	0.100	0.500
Progesterone***	0.010	0.025
Testosterone***	0.010	0.025
Vinclozolin*	0.010	0.025

* Pesticide
** BNA Compound
*** Hormone

5.4 Laboratory Quality Control

Trace organic laboratory QC samples will include method blanks, spike blanks, matrix spikes, matrix spike duplicates, and surrogates. Method blanks, spike blanks, matrix spikes, and matrix spike duplicates will be analyzed at a frequency of one per analytical batch or a minimum of one per 20 analytical samples. Surrogates are analyzed with every analytical sample.

- A **method blank** is an aliquot of a clean reference matrix, such as deionized, distilled water for water samples, which is processed through the entire analytical procedure. Analysis of method blanks is used to evaluate the levels of contamination that might be associated with the processing and analysis of samples. Method blank results should be "less than the MDL" for all target analytes.
- A **spike blank** is an aliquot of clean reference matrix, such as deionized distilled water for water samples, to which a known concentration of one or more target analytes has been added. The spiked aliquot is processed through the entire analytical procedure. Analysis of the spike blank is used as an indicator of method performance and can also be used in conjunction with matrix spike results as an indicator of sample matrix effects. Control limits are based on the percent recovery of the spiked compounds.
- A **matrix spike (MS)** is a known concentration of one or more target analytes, which is introduced into a second aliquot from one analytical sample. The spiked sample is processed through the entire analytical procedure. Analysis of the MS is used as an indicator of sample matrix effect on the recovery of target analytes. Control limits are based on the percent recovery of the spiked compounds.

- A **matrix spike duplicate (MSD)** is a known concentration (same as the MS) of target analytes, which is introduced into a third aliquot of the same analytical sample. The spiked sample is processed through the entire analytical procedure. Analysis of the MSD is used as an indicator of sample matrix effect on the recovery of target analytes as well as method precision. The relative percent difference (RPD) between the MS and MSD results is calculated, however, control limits are not maintained. The RPD for MS/MSD results is, instead, reviewed during the data validation and analysis process to evaluate any data quality issues arising from questions of analytical precision.
- A **surrogate** is a known concentration of one or more non-target analytes which is added to every sample (both analytical and QC samples) prior to extraction. Analysis of surrogates is used as an indication of method or matrix bias for target compounds on a sample-specific basis. Surrogate compounds are selected that behave in a similar manner to target analytes. Control limits are based on the percent recovery of the surrogate compounds.

Table 8 summarizes the control limits for trace organic laboratory QC samples.

Table 8
Trace Organic Laboratory QC Samples and Control Limits

QC Sample	Chlorinated Pesticides and PCBs	BNAs/Atrazine	Misc. Endocrine Disrupting Comp.
Method Blank Result	All compounds <MDL	All compounds <MDL	All compounds <MDL
Spike Blank Recovery	23 to 139%*	9 to 127%*	50 to 150%
MS/MSD Recovery	23 to 139%*	9 to 127%*	50 to 150%
MS/MSD RPD	Not Applicable	Not Applicable	Not Applicable
Surrogate Recovery	50 to 150%	10 to 141%*	50 to 150%

*Low to high range of all compounds used for surrogates or spikes.

QC sample results that exceed control limits will be evaluated to determine appropriate corrective actions. Samples will typically be reanalyzed if unacceptable QC results indicate a systematic problem with the overall analysis, and if sufficient sample matrix is remaining and the analytical holding time has not expired. Unacceptable QC results caused by a particular sample or matrix will not require reanalysis unless an allowed method modification would improve the results. Analytical results that are outside of QC control limits will be qualified and flagged according to procedures outlined in Section 7.

6 IMMUNOASSAY TESTING FOR ESTRADIOL AND ETHINYLESTRADIOL

Enzyme-linked immunosorbent assay (ELISA) testing will be performed on water samples to measure concentrations of two hormones, estradiol and ethinylestradiol. Trace organic GC-MS results will be used to evaluate results from the ELISA testing and to assess the potential of using these relatively low-cost, rapid assays as a reliable tool for measuring concentrations of hormonal compounds in natural waters.

Data comparability between GC-MS and ELISA results will be evaluated by the data comparability guidelines established in EPA, 1996. Data comparability analysis will include development of statistical correlation between GC-MS and ELISA results. Development of statistically-valid correlation factors will be dependent on having a sufficient number of results greater than the MDL in the data set.

6.1 Estradiol

The quantitative analysis of estradiol (17b-estradiol) in water samples will employ the American Laboratory Products (ALPCO) Estradiol Plate Kit®. This estradiol ELISA kit is based on the competition principal in which an unknown amount of estradiol present in the sample and a fixed amount of estradiol conjugated with horse-radish peroxidase (HRP) compete for a fixed number of binding sites to polyclonal estradiol antiserum coated onto microtiter wells.

After a two-hour incubation, the microtiter plate is washed to remove the unbound HRP conjugate. A substrate is then added and the plate incubated for 15 minutes. The enzyme-substrate reaction is stopped with acid and the color that has developed in the wells is measured in a colorimeter at 450 nanometers (nm). The color measurement is proportional to the bound enzyme conjugate and inversely proportional to the estradiol concentration in the water sample.

This method measures the concentration of free, unconjugated estradiol in natural water samples. The estradiol ELISA test has a reported MDL of 0.020 μg/L in both fresh and salt water. Samples may be concentrated, using EPA Method 3535A (SW846) Solid-Phase Extraction (SPE) technique, to detect low-level ambient concentrations of estradiol. The maximum concentration that can be measured without dilution is 0.50 μg/L, which is the highest standard on the calibration curve.

6.2 Ethinylestradiol

The qualitative analysis of ethinylestradiol in water samples will employ the Ridascreen Ethinylestradiol Plate Kit®. This ethinylestradiol ELISA kit uses a double antibody system. The anti-ethinylestradiol antibodies are added to the wells together with the ethinylestradiol-enzyme conjugate and the test sample. The anti-ethinylestradiol antibodies bind to a fixed number of immobilized sheep antibodies in the wells. A fixed amount of ethinylestradiol-enzyme conjugate and the unknown amount of ethinylestradiol in the sample compete for the binding sites on the anti-ethinylestradiol antibodies.

After a two-hour incubation, the microtiter plate is washed to remove the unbound conjugate. A substrate and chromogen are then added and the plate is incubated for 30 minutes. Bound enzyme conjugate converts the colorless chromogen into a blue product. The enzyme-

substrate reaction is stopped with acid which leads to a color change from blue to yellow. The color that has developed in the wells is measured in a colorimeter at 450 nm. The color measurement is inversely proportional to the ethinylestradiol concentration in the water sample.

This method measures the concentration of free, unconjugated ethinylestradiol in natural water samples. The ethinylestradiol ELISA test has a reported MDL of 0.030 μg/L in both fresh and salt water. Samples may be concentrated, using SPE, to detect low-level ambient concentrations of ethinylestradiol. SPE concentration factors will be adjusted to bring measurements within the range of standards. The concentration factors applied will be reported with each set of sample results. The maximum concentration that can be measured without dilution is that of the highest standard, 1.08 μg/L.

6.3 Immunoassay Testing QC Procedures

The following QC procedures will be used for both estradiol and ethinylestradiol methods. The particular QC samples analyzed will depend on whether the SPE technique is utilized for a particular batch of samples.

- A **method blank** is an aliquot of a clean reference matrix, which is processed through the entire analytical procedure when the SPE technique is used. Analysis of method blanks is used to evaluate the levels of positive bias that might be associated with the processing and analysis of samples. Method blank results should be “less than the MDL” for each target analyte.
- A **negative control** is included with each ELISA kit and is analyzed in duplicate with each batch of samples. The negative control is not processed through the SPE technique and is equivalent to a method blank for samples where the SPE technique is not used. Negative control results should be "less than the MDL" for each target analyte.
- A **spike blank** is an aliquot of clean reference matrix, to which a known concentration of the target analyte has been added. The spiked aliquot is processed through the entire analytical procedure, when the SPE technique is used. Analysis of the spike blank is used as an indicator of method performance and can be used in conjunction with matrix spike results as an indicator of sample matrix effects. Control limits are based on the percent recovery of the spiked compounds.
- A **matrix spike (MS)** is a known concentration of one or more target analytes, which is introduced into a second aliquot from one analytical sample. The spiked sample is processed through the entire analytical procedure when the SPE technique is used. Analysis of the MS is used as an indicator of sample matrix effect on the recovery of target analytes. Control limits are based on the percent recovery of the spiked compounds.
- A **positive control** is a separate portion of the mid-point calibration standard that is analyzed in duplicate with each batch of samples. The positive control is not processed through the SPE technique. Both the percent recovery of the positive control and the difference between the duplicate measurements are evaluated.

7 DATA VALIDATION, REPORTING, AND RECORDKEEPING

Data validation is critical for evaluating how well analytical data meet project DQOs. Data validation is performed, at some level, during several steps in the process of sample analysis. All trace organic instrument analytical data will be entered into King County's Laboratory Information Management System (LIMS).

7.1 Data Validation

Laboratory analytical data are reviewed, first by the primary analyst and then by a peer reviewer. Analytical data are reviewed for completeness and QC sample data are reviewed for compliance with project and method QA/QC requirements. If there are any QC failures at this point, corrective action may be taken or qualifier flags applied to the data.

A laboratory project manager (LPM) will provide the next data review step, at a project level. The LPM will verify the completeness of an entire data set and report any QC failures or anomalies. A project data validator will provide a final review of the data to ensure they meet the project DQOs. Data then will be reported in a variety of formats, depending on project needs.

7.2 Data Reporting

Immunoassay results will be reported as quarterly narrative reports of results and associated QC testing. Sample and QC results will be submitted to the LPM and data validator as electronic files in Excel® format until such time that the data are accessible through LIMS. At present, the necessary programming and software testing required to make immunoassay data accessible through LIMS is expected to be completed in August 2003.

All laboratory analytical data are maintained *in perpetuity* on LIMS. Data may be viewed on-line in LIMS by King County personnel only. Project data may also be downloaded from LIMS into a hard copy format using Microsoft Excel®. Analytical data will be reported on a routine basis in Excel® format along with an accompanying QA/QC review narrative.

Laboratory analytical data may be stored with data qualifier flags indicating QC failures. The flag "B" is used to indicate possible laboratory contamination of a sample and is applied when a target analyte is detected in the laboratory method blank. Sample results that are less than five times the concentration detected in the method blank will be qualified with a "B" flag. The flag "H" is used to indicate a sample handling condition that did not meet method requirements. Handling conditions may include an improper sample container, improper preservation of the sample, or an exceedence of the method-specific holding time. The flag "E" may be applied to sample data at the discretion of the laboratory analyst or peer reviewer, should control limits on one or more QC samples not be met. The flag "E" indicates that sample data should be viewed as *estimated*.

Analytical results from field blanks and field replicates will be reviewed to evaluate their impact on the quality and usability of sample analytical data. Results from field QC samples will not be used to flag sample analytical data but will be taken into consideration during final data review, analysis, and reporting.

7.3 Recordkeeping

Hard-copy field notes, raw analytical data, and any other hard-copy project information will be stored according to standard King County Environmental Laboratory practices for a period of ten years.

7.4 Special Data Qualification for BNA Compounds and Atrazine

Every reported compound will be evaluated with a continuing calibration standard for each 12-hour shift. The acceptable continuing calibration recovery is 80 to120% for every target analyte. Sample data for any detected target analyte for which the percent recovery is greater than 120% will be qualified with an "L" flag. Sample data for any detected target analyte for which the percent recovery is less than 80% but equal to or greater than 50% will be qualified with a "G" flag. If the percent recovery for any target analyte is less than 50%, either corrective action will be taken to meet the 50% criterion and the samples rerun or associated sample data for the target analyte will be qualified with a "R" flag.

These qualifier flags indicate that:

- L – the reported value may be biased high, based on continuing calibration information;
- G – the reported value may be biased low, based on continuing calibration information;
- R – the reported data for this target analyte are rejected and should not be used in any capacity, based on continuing calibration information.

Any target analyte reported at a concentration between the MDL and RDL will be qualified "<RDL." If this value is less than the lowest concentration in the calibration curve, the sample data will also be qualified with an "E" flag, indicating that the reported concentration is estimated.

8 REFERENCES

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